

**IN VITRO CYTOTOXIC ACTIVITY OF ETHYL ACETATE FRACTIONS
OF MARINE SPONGE *Calthropella* sp. COLLECTED FROM
KRAKAL BEACH GUNUNGKIDUL**

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ABSTRACT

A research on isolation, *in vitro* cytotoxic assay, and structure identification of ethyl acetate fractions of sponge *Calthropella* sp. from Krakal Beach Gunungkidul had been carried out. This study is aimed to investigate the cytotoxic activity of the ethyl acetate fractions isolated from *Calthropella* sp. marine sponge collected from Krakal Beach Gunung Kidul. The extraction was done by using dichloromethane:methanol (2:1, v/v). The dichloromethane extract was partitioned in ethyl acetate:water (3:2, v/v). The interesting ethyl acetate extract was then separated by column chromatography. Toxicity test from ethyl acetate extract was carried out by Brine Shrimp Lethality Test (BSLT) method, while the *in vitro* cytotoxic assay was performed by MTT assay against breast adenocarcinoma cancer (MCF-7), lung cancer (H-460), and hepatocarcinoma liver cancer cell lines (HepG-2). The active fractions were then analyzed by High Resolution Liquid Chromatography-Mass Spectrometry (HR LC-MS).

The ethyl acetate extract was obtained as yellowish brown (217.50 mg). This extract gave LC₅₀ value 105.38 µg/mL from BSLT, which is considered to be toxic and has pharmacological activity. In MTT *in vitro* cytotoxic assay, fraction 6 has strong activity against MCF-7 cell lines (IC₅₀ 22.84 µg/mL), but showed weak activity against H-460 and HepG-2 cell lines IC₅₀ value 323.85 and 335.46 µg/mL respectively. Fraction 7 has strong cytotoxic activity against MCF-7 cell lines (IC₅₀ 1.92 µg/mL), but showed moderate cytotoxicity against H-460 and HepG-2 cell lines with IC₅₀ value 165.13 and 53.01 µg/mL respectively. Fraction 6 and 7 showed promising cytotoxicity on MCF-7 cell lines with IC₅₀ < 30 µg/mL. Based on LC-MS analyses, it is strongly predicted that active fraction 6 consist of 4'-N-methyl-5'-hydroxystaurosporine, 24-methyleneergost-4-ene-3-one, and one new compound. Fraction 7 is predicted to consist bengamide Q, clavepictine A, 4'-N-methyl-5'-hydroxystaurosporine, biemnic acid, carteriofenone A, and one new compound.

Keywords: *Calthropella* sp., MTT assay, MCF-7, H-460, HepG-2

**UJI SITOTOKSISIK IN VITRO FRAKSI ETIL ASETAT SPONS LAUT
Calthropella sp. ASAL PANTAI KRAKAL GUNUNGKIDUL**

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INTISARI

Telah dilakukan penelitian tentang isolasi, uji sitotoksik *in vitro*, dan identifikasi struktur dari fraksi etil asetat spons *Calthropella* sp. asal Pantai Krakal Gunungkidul. Penelitian ini bertujuan untuk mengevaluasi aktivitas sitotoksik fraksi etil asetat hasil isolasi dari sponge *Calthropella* sp. asal Pantai Krakal Gunung Kidul. Isolasi dilakukan dengan metode ekstraksi menggunakan diklorometana:metanol (2:1, v/v) sebagai pelarut. Ekstrak diklorometana selanjutnya dilakukan partisi dalam pelarut etil asetat:air (3:2, v/v). Ekstrak etil asetat yang diperoleh dipisahkan dengan kromatografi kolom. Uji toksisitas ekstrak etil asetat dilakukan dengan metode *Brine Shrimp Lethality Test* (BSLT) dan uji sitotoksik *in vitro* dilakukan dengan metode MTT terhadap kultur sel kanker payudara (MCF-7), sel kanker paru-paru (H-460), dan sel kanker hati (HepG-2). Analisis senyawa dari fraksi aktif dilakukan dengan *High Resolution Liquid Chromatography-Mass Spectrometry* (HR LC-MS) untuk menentukan massa molekul senyawa-senyawa penyusunnya.

Hasil ekstraksi diperoleh ekstrak kasar etil asetat berwarna kuning kecoklatan sebesar 217,5 mg. Hasil pemisahan diperoleh 8 fraksi dan uji toksisitas ekstrak etil asetat menunjukkan nilai LC_{50} 105,38 $\mu\text{g/mL}$, memberikan indikasi toksik dan memiliki aktivitas farmakologi. Berdasarkan hasil uji sitotoksik *in vitro* dengan metode MTT, fraksi 6 menunjukkan aktivitas sitotoksik kuat terhadap sel MCF-7 (IC_{50} 22,84 $\mu\text{g/mL}$), tetapi sitotoksiknya melemah terhadap sel H-460 dan HepG-2 dengan nilai IC_{50} 323,85 dan 335,46 $\mu\text{g/mL}$ berturut-turut. Fraksi 7 menunjukkan aktivitas sitotoksik kuat terhadap sel MCF-7 (IC_{50} 1,92 $\mu\text{g/mL}$) dan sitotoksik sedang terhadap sel H-460 dan HepG-2 dengan nilai IC_{50} 165,13 dan 53,01 $\mu\text{g/mL}$ berturut-turut. Fraksi 6 dan 7 disimpulkan menunjukkan potensi sitotoksik terhadap sel MCF-7, dengan memiliki nilai $IC_{50} < 30 \mu\text{g/mL}$. Analisis LC-MS mengindikasikan kuat bahwa senyawa hasil isolasi dari fraksi aktif 6 terdiri dari 4'-N-metil-5'-hidroksistaurosporin, 24-metileneergos-4-en-3-on, dan satu senyawa baru. Fraksi 7 diprediksi kuat mengandung bengamida Q, klafepiktin A, 4'-N-metil-5'-hidroksistaurosporin, asam biemnik, karteriofenon A, dan satu senyawa baru.

Kata kunci: *Calthropella* sp., MTT, MCF-7, H-460, HepG-2